Claims 1-3, 5, 6, 8, 9, 13-16 and 18 are pending. New claims 19 and 20 are added herein. Claims 4, 7, 11, 12, 15 and 17 are cancelled without prejudice. Claims 1, 5, 6, 8, 9, 13, 14, and 16 are amended herein.

The amendment to claim 1 places the limitation of claim 4, that the mammal "has made an immune response to said immunogenic polypeptide," into claim 1. This amendment adds no new matter.

New claim 19 recites all limitations of claim 1 as pending prior to this amendment, plus the limitation of cancelled claim 7, that "prior to introduction of the cell into the mammal the expression of the polypeptide is inhibited in vitro." This new claim therefore adds no new matter.

New dependent claim 20 adds the limitation "wherein after introduction of the cell into the mammal, expression of the polypeptide reaches a maximum level in the mammal after two days" to new claim 19. This language is taken directly from cancelled claim 7 and therefore adds no new matter.

The amendments to claims 5, 6, 8 and 9 are made in order to correct the dependency of these claims after the amendment of claim 1 and the addition of new claim 19.

The specification is objected to because the abstract allegedly contains legal phraseology such as "comprising" and "said." Applicants have amended the abstract to remove the terms "comprising" and "said", replacing them with "entailing" and "the", respectively. Applicants submit that the amendments introduce no new matter.

The specification is also objected to because the description of Fig. 2A, 2B and 3 allegedly does not describe Fig. 3 individually and because FACS data displayed in Fig. 2A, 2B and 3 are allegedly not described adequately. Applicants have amended the specification to describe the Figures 2 and 3 separately and to provide more detail in the description of the figures. Applicants submit that the description of Figures 2A and 2B was copied directly from the text of the specification at page 26, line 29 through page 27, line 9, and the description of Figure 3 was copied directly from the text at page 27, line 33 to page 28, line 13. The amendment adds no new matter.

Rejections under 35 U.S.C. §112, first paragraph:

Claims 1-9 and 11-18 are rejected under 35 U.S.C. §112, first paragraph for alleged overbreadth. The Office Action states that while the specification is enabling for a method of regulating the expression of a nucleic acid sequence encoding an immunogenic peptide in vitro, the specification "does not reasonably provide enablement for a method of regulating the expression of a nucleic acid sequence using a non-tetracycline regulatable system, or regulating the nucleic acid in vivo." Applicants respectfully disagree.

First, Applicants note that the claims are limited to the use of a system regulatable by tetracycline or an analog thereof. Therefore, the alleged non-enablement of non-tetracycline-mediated regulation is moot.

The Examiner expressed doubt regarding the utility of the claimed invention for anything other than gene therapy in humans. Applicants submit that the prior art demonstrates the utility of regulated expression of marker genes in vivo. For example, cells expressing a marker polypeptide are used extensively for cell lineage studies. The ability to turn marker gene expression on (or off) with precision was recognized as a useful attribute in the pre-filing date literature. For example, Furth et al., 1994, Proc. Natl. Acad. Sci. U.S.A. 91: 9302-9306 (Exhibit A), and Hennighausen et al., 1995, J. Cell. Biochem. 59: 463-472 (Exhibit B) describe studies using the tet operator system to modulate gene expression in transgenic mice. Tissue specific and temporal regulation of the marker transgenes is demonstrated.

The utility of tightly controlled polypeptide expression for uses in addition to gene therapy is recognized in the specification at page 32, lines 17 and 18, which state "[t]he ability to regulate easily the expression of TCR molecules at defined levels could be relevant to studies of the stoichiometry of the T cell activation process." In this situation, the prior immune response of the mammal to the immunogenic polypeptide would have had the effect of generating a population of armed effector T cells that are primed to become activated by presentation of antigens derived from this immunogenic polypeptide. Enablement for this specific use and others within the ability of those ordinarily skilled in the art is provided in the specification and in the prior art as follows.

With regard to the enablement for regulating a nucleic acid sequence in vivo, the Examiner states that the specification does not provide specific guidance regarding the parameters required to regulate expression of a protein in vivo such that an enabled use is

obtained. The prior art contains numerous examples of the use of marker polypeptides (which are most often foreign to mammals and therefore immunogenic) in cells administered in vivo. In each instance, parameters including vectors, promoters and dosages of cells necessary to obtain the desired expression of the marker polypeptide are described. One common use of marker polypeptides is to study cell lineages. For example, Pin & Merrifield (1997 (Aug), Dev. Biol. 188: 147-166; Exhibit C) describe lineage studies of rat myoblasts marked with a LacZ reporter gene and injected into regenerating rat muscle.

Allay et al. (1997 (Aug.), Hum. Gene Ther. 8: 1417-1427; Exhibit D) describe studies using LacZ-marked human marrow-derived mesenchymal progenitor cells to study the differentiation potential of the cells in vivo.

Rosenbluth et al. (1997 (Sept), Exp. Neurol. 147: 172-182; Exhibit E) describe the injection of LacZ-marked mouse glia into immunosuppressed rats with experimental traumatic spinal cord injuries. The transplanted cells became established and expressed processes consistent with myelin segments.

Tajbakhsh et al. (Exhibit A, 1996, Dev. Dyn. 206: 291-300; Exhibit F) describes cell lineage studies using embryonic stem cells transfected with a lacZ reporter driven by myf-5 regulatory sequences. The cells were injected into developing embryos and expression of the marker polypeptide was characterized in order to examine myf-5 driven expression throughout the embryo.

Zhang & Goldman (1996, Neuron 16: 47-54; Exhibit G) describe the use of a lacZ marker gene to examine the origins of cerebellar interneurons.

Each of the above references teaches vectors, promoters, and cell dosages useful for their respective research uses. Using these and other studies in the prior art, one skilled in the art can determine the values for these parameters without undue experimentation.

Applicants have previously provided prior art references teaching the necessary parameters for tetracycline-regulated gene expression in vivo (e.g., Shockett et al., Hofmann et al.), and the Furth et al. and Hennighausen et al. references cited herein (Exhibits A and B) add to those teachings. Given the guidance in these references and in the specification, one of skill in the art would be able to use the methods of the invention, for example, to examine cell lineages at different stages of development or maturity. The invention as described in the specification enables a researcher, for example, to use an immunogenic marker polypeptide, e.g., LacZ, to

study differences in differentiation, proliferation, gene expression, or cellular activation potentials (e.g., T cell activation) for a given cell type over time, in the same animal, without interference caused by the immune response to the marker polypeptide. Using the methods of the invention, cells may be administered and permitted to proliferate and/or differentiate for a chosen time before turning on the expression of the marker polypeptide by altering the tetracycline concentration present in the animal's system. Any additional information regarding vectors, promoters or dosages of vectors, cells or tetracycline will be specific to the given study and can, using guidance in the specification and in the prior art, be empirically determined by one of skill in the art without undue experimentation. Therefore, Applicants submit that there are enabled, practical uses of the invention that are not necessarily therapeutic.

In view of the above, the specification and prior art (e.g., Examples 1 and 2) therefore enable the methods of claim 1 and claim 19. More specifically, the specification (e.g., Examples 1 and 2) and prior art (e.g., Shockett et al., Hofmann et al., and Exhibits A and B) teach how to make a cell comprising a vector comprising a nucleic acid encoding an immunogenic polypeptide, operably linked to a tetracycline-regulatable promoter. The specification (e.g., on page 20, in the section titled "Dosage and Administration) and the prior art (e.g., Exhibits C-F) teach how to administer a cell comprising such a vector to a mammal. The specification (e.g., in the same "Dosage and Administration section") and prior art (e.g., Exhibits A and B) teaches how to alter the concentration of tetracycline or analog thereof to which the cell is exposed so as to achieve in the mammal expression of the nucleic acid sequence. It is submitted that the operability of the claimed invention is thus demonstrated as of the Applicants' filing date. All that remains to satisfy the enablement of the claimed invention is to administer the cells to a mammal that has already made an immune response to the immunogenic polypeptide. It is submitted that it is straightforward and easy to determine, using techniques well known in the art at the time of filing whether or not a mammal has raised an immune response to a given polypeptide. Therefore, Applicants submit that the method of claim 1 is enabled by the specification and the prior art.

Similarly, the specification describes the inhibition of the tet-regulated expression in vitro prior to administration of the cells to a mammal (see, e.g., page 9, first full paragraph). Therefore, new claim 19 is also enabled by the specification and knowledge available in the prior art.

The Office Action also states that "the specification does not teach how to target the tetracycline to cells administered to a mammal as claimed in the instant invention." Applicants submit that there is no requirement for targeting of the drug to the administered cells, either in fact or in the claims. Tetracycline is administered systemically and becomes widely distributed throughout the tissues of the body. As such, there is no need for "targeting" the drug. One illustration of this is the method embodied in claim 8. In that method, expression of the immunogenic polypeptide is inhibited in vitro by exposure of the cell to tetracycline or an analog thereof. By administering the cells with the tet-inhibited construct to a mammal and then withdrawing tetracycline (or analog), the expression of the polypeptide is induced in the mammal after 2 days following the removal of tetracycline or analog. Clearly, a method that uses removal of the systemic drug does not require targeting of the drug. The same is also true of the converse situation, in which transgene expression is induced by the addition of the drug.

Applicants respectfully request the withdrawal of the §112, first paragraph rejection of claim 1 and submit that new claim 19 is not susceptible to rejection on the same grounds.

The Office Action states that the specification does not enable regulating the expression of a polypeptide by altering the concentration of regulatory drug after the cell has been administered. The Office Action also states that the claims are not limited to transfecting the cells in vitro, culturing the cells in vitro with tet, removing tet in vitro and administering the cells to a mammal," concluding that "the method of regulating the expression is not enabled as broadly claimed." Applicants respectfully disagree.

The specification describes the systems in which the presence of tet induces (tet-on) or inhibits (tet-off) the expression of a tet operator-linked sequence (see for example, page 12, line 15 to page 13, line 16). The embodiment using the tet-off system is enabled by the description in Example 1 (see page 27, line 15 to page 29, line 10), in which tet exposure maximally inhibited expression by 24 hours. Administering the inhibited cells to an animal with no serum tetracycline will induce the expression from these constructs in these cells. This is one embodiment of a method entailing "altering the concentration of regulatory drug after the cell has been administered." In this embodiment, there is no need to teach how to administer or alter tetracycline in the mammal. It is simply not administered at all.

The specification, as noted above, also teaches in vivo dosages of tet on page 20 (dosages adjusted to achieve tet serum concentrations of 0.05 to 1.0 micrograms per ml). Applicants

submit that the concentration of tet necessary to repress *or activate* expression in vitro will mirror the effective concentration necessary in vivo for the same effect. One of ordinary knowledge of the pharmacology of tetracycline can readily adjust the serum concentration of tet by adjusting the amount administered and the schedule of its administration. The specification and knowledge in the prior art therefore provide sufficient information to enable one of skill in the art to alter the concentration of tetracycline or analog thereof in vivo before, during and after administration of cells.

The Office Action repeats the prior rejection of claims 4-6 for alleged non-enablement of regulating gene expression in a mammal that has made an immune response to the immunogenic polypeptide prior to administration of the cell. The Office Action states that there is not adequate guidance as to what applicants consider the appropriate immune response or how to detect it. The Office Action also states that the claims do not provide a nexus between a method of regulating a gene which results in a desired immune response.

Applicants submit that detection of immunity to a given antigen is a standard methodology. Standard methods include agglutination assays, ELISAs and FACS analyses to identify lymphocytes specific for a known antigen. Under U.S. patent law, one need not describe those aspects of an invention that are well known in the art. Hybritech, Inc. v. Monoclonal Antibodies, Inc., 802 F.2d 1367 (Fed. Cir. 1986). As such, it is not necessary for the specification to include detailed methods for the detection or measurement of immunity to the polypeptide one wishes to express. Also, Applicants stress again that the claimed methods do not aim to *induce* an immune response, but rather aim to produce an immunogenic polypeptide in vivo *despite* the immune response. Applicants therefore respectfully request the withdrawal of this rejection.

The Office Action rejects claims 7-9 as allegedly lacking enablement because there is no nexus between the preamble and the body of the claims. The Office Action states that the inhibition of the polypeptide in vitro as recited in claim 7 is not regulating the expression of a polypeptide in a mammal as in the preamble of claim 1. The Office Action also states that "claim 7 does not recite when the tetracycline is removed to allow expression; therefore, the claim encompasses regulating the expression of a protein in a mammal by altering tetracycline levels while it is within the mammal" and concludes "however, the specification does not

provide any guidance how to alter tetracycline levels while the cells are in the mammal. Applicants respectfully disagree.

Claim 7 has been cancelled, rendering this rejection of that claim moot. However, the limitations of claim 7 have been retained in new claims 19 and 20. The fact that the claims may encompass regulating the expression of a protein by altering the concentration of tetracycline in the mammal does not mean that the claims are not enabled. The specification does provide guidance regarding serum tetracycline or tetracycline analog concentrations. As discussed above, those skilled in the art presumably understand the basic principles of pharmacology, and can adjust the amount and timing of drug administration to achieve a given serum level, including both maintaining a desired level and achieving the substantial absence of the drug by removing it. Applicants therefore respectfully request the withdrawal of this rejection of claims 7-9.

The Office Action rejects claim 13 as allegedly lacking enablement because it is allegedly unpredictable how to regulate gene expression in vivo using viral vectors. Applicants respectfully disagree. Applicants submit that the introduction of gene sequences to cells using viral vectors is extremely well established and far from unpredictable. Applicants stress that the claimed methods do not call for the introduction of foreign gene sequences into cells in vivo, but rather call for the use of a viral vector to introduce a tet-regulated transgene construct to cultured cells. Therefore, applicants need not enable the regulation of gene expression in vivo using viral vectors. Applicants respectfully request the withdrawal of this rejection of claim 13.

Rejections under 35 U.S.C. §112, second paragraph:

Claims 1-9 and 11-18 are rejected under 35 U.S.C. §112, second paragraph for alleged indefiniteness.

Claims 1-9 and 11-18 are allegedly indefinite because claim 1 is directed to regulating the expression of a nucleic acid sequence but only results in expression of a nucleic acid sequence. Applicants respectfully disagree. Applicants submit that the claimed method results in "achieving in said mammal expression of said nucleic acid sequence *as permitted in the presence or absence of tetracycline or an analog thereof.*" The italicized language is descriptive of regulation. Expression as permitted in the presence or absence of a drug is regulated expression

dependent on the presence or absence of the drug. Applicants respectfully request the withdrawal of this §112, second paragraph rejection.

Claim 18 is rejected as allegedly indefinite because the phrase "so as to regulate" is an intended use and may not occur. Applicants submit that claim 18 as amended recites "thereby regulating the expression of the coding sequence." The amendment is believed to obviate the rejection.

Claims 1-9 and 11-13 are allegedly indefinite because the preamble of claim 1 states that the polypeptide is "immunogenic in the mammal," but the body of claim 1 does not require that the polypeptide is "immunogenic." Applicants submit that claim 1 as amended recites, within the body of the claim, "into a mammal that has made an immune response to said immunogenic polypeptide." Applicants submit that the amendment obviates this §112, second paragraph rejection and respectfully request withdrawal of the rejection.

Claims 1-9, 11-13 and 18 are rejected as allegedly indefinite for use of the phrase "altering the concentration of tetracycline... to which the cell/leukocyte is exposed" because the phrase allegedly relates to the step of administering the cells. It is allegedly unclear whether the concentration of tet is altered before or after administering the cells to the mammal. Applicants submit that these are improper grounds for an indefiniteness rejection. While the tet concentration will most often be altered before or concurrent with administration to a mammal, the claim is *intended* to encompass the situation in which the concentration of tet is altered *before or after* such administration. Therefore, Applicants submit that the claim, which can encompass either situation, is not indefinite, and respectfully request withdrawal of the rejection.

Claims 4-9 are rejected as allegedly indefinite for use of the terms "said polypeptide" or "the polypeptide" because it is allegedly unclear whether the terms refer to an "immunogenic polypeptide" as in the preamble of claim 1 or a generic polypeptide in the body of the claim. Applicants submit that the amendment of "the polypeptide" and "said polypeptide"to "said immunogenic polypeptide" in each of claims 5, 6, 8 and 9 makes clear that the term refers to the same immunogenic polypeptide in each case (the cancellation of claims 4 and 7 renders moot the rejection of these claims). Applicants respectfully request withdrawal of this rejection.

Claim 1 is rejected under §112, second paragraph because the term "the mammal" in lines 2 and 3 allegedly lacks antecedent basis. Applicants submit that the amendment of the claim obviates this ground of rejection.

Claim 17 is rejected as allegedly indefinite because the phrase "said nucleic acid coding sequence" lacks proper antecedent basis. Applicants submit that the cancellation of claim 17 renders this rejection moot.

Claim 18 is rejected as allegedly indefinite because the phrase "the isolated leukocyte" lacks proper antecedent basis. Applicants submit that the phrase "the isolated leukocyte" does not occur in the claim. Clarification or withdrawal of the rejection is requested.

Rejections under 35 U.S.C. §102:

A. The Shockett et al. reference

Claims 1, 14, 16 and 17 are rejected under 35 U.S.C. §102(b) as allegedly being anticipated by Shockett et al. The Office Action states that Shockett et al. teaches transducing fertilized eggs with a vector encoding luciferase under the control of the tet operator, and implanting them into pseudopregnant females. The Office Action concludes that this is the equivalent of introducing a cell into a mammal as claimed and that the reference therefore anticipates the claimed invention. Applicants respectfully disagree.

Shockett et al. does not teach a method comprising "introducing, into a mammal that has made an immune response to [a] polypeptide, a cell comprising a vector comprising a nucleic acid encoding [the] polypeptide," as required by amended claim 1. There is no teaching in the Shockett et al. reference regarding the introduction of sequences encoding luciferase into a mammal that has already raised an immune response to luciferase. Therefore, Shockett et al. does not anticipate claim 1 as amended.

Shockett et al. does not teach "an isolated leukocyte transformed with a nucleic acid sequence encoding a polypeptide which is immunogenic to a mammal," as required by claim 14 as amended. A fertilized egg is not a leukocyte, nor is an NIH3T3 fibroblast, also mentioned in the Office Action as being taught by Shockett et al. Therefore, Shockett et al. does not anticipate claim 14 as amended. For the same reason, the Shockett et al. reference does not anticipate amended claim 16, which recites a "composition comprising a plurality of a leukocyte of claim 14 and a physiologically acceptable diluent. The rejection of claim 17 over Shockett et al. is made moot by the cancellation of claim 17.

In view of the above, Applicants respectfully request the withdrawal of the §102(b) rejection of claims 1, 14 and 16 over Shockett et al.

Applicants further submit that the Shockett et al. reference does not anticipate new claims 19 or 20. First, Shockett et al. does not teach introducing a cell to a mammal wherein prior to introduction of the cell into the mammal the expression of the polypeptide is inhibited in vitro, as also required by new claim 19. The reference also does not teach altering the concentration of tetracycline or an analog thereof to which the cell is exposed in the mammal, wherein expression of the polypeptide reaches a maximum level in the mammal after 2 days, as required by new dependent claim 20. Because the Shockett et al. reference does not teach any of these elements required by new claims 19 and 20, the claims are not anticipated by the reference.

B. The Hoffmann et al. reference

Claims 14-17 are rejected under 35 U.S.C. §102(b) as allegedly being anticipated by Hoffmann et al. The Office Action states that the reference teaches transfecting lymphocytes with a retroviral vector encoding LacZ operatively linked to the tet operator and also encoding the tetR-VP16. The Office Action states that "the lymphocytes taught by Hoffmann are 'leukocytes' as claimed." Applicants disagree.

Applicants submit that Hoffmann et al. teaches only the transfection of myoblasts, which are muscle cell progenitors, not leukocytes. Applicants respectfully suggest that the Examiner may have been confusing myoblasts, the muscle cell progenitors, with "myeloblasts," which are in the lymphoid lineage. Because Hoffmann et al. does not teach the transfection of any leukocyte, the reference cannot teach an isolated leukocyte transformed with a nucleic acid sequence encoding a polypeptide which is immunogenic to a mammal, as required by claim 14 as amended or by claim 16 that depends from it. The rejection of claim 17 over Hoffmann et al. is made moot by the cancellation of claim 17.

In view of the above, Applicants respectfully request the withdrawal of the §102(b) rejection of claims 14-16 over Hoffmann et al.

Applicants further submit that the rejection in view of Hoffmann et al. applied to claims 14-17 does not apply to new claims 19 and 20. First, Hoffmann et al. does not teach introducing a cell to a mammal as required by new claim 19. Second, Hoffmann et al. does not teach introducing a cell to a mammal wherein prior to introduction of the cell into the mammal the expression of the polypeptide is inhibited in vitro, as also required by new claim 19. The reference also does not teach altering the concentration of tetracycline or an analog thereof to

which the cell is exposed in the mammal, wherein expression of the polypeptide reaches a maximum level in the mammal after 2 days, as required by new claim 20. Because the Hoffmann et al. reference does not teach any of these elements required by new claims 19 and 20, the claims are not anticipated by the reference.

C. The Cooke et al. reference

Claims 14-17 are rejected under 35 U.S.C. §102(a) as allegedly being anticipated by Cooke et al. The Office Action states that Cooke et al. teaches transfecting human T cell lines and mouse macrophages with a vector encoding Nef operably linked to a CMV/tetracycline operator promoter and a vector encoding tTA. The Office Action concludes that because Nef is a foreign immunogenic protein, T cells are leukocytes, and culture medium "is considered a physiologically acceptable diluent," claims 14-17 are anticipated by the reference. Applicants respectfully disagree.

Applicants submit that Cooke et al. does not teach the transfection of T cell or macrophage lines with a nucleic acid sequence encoding a polypeptide which is immunogenic to a mammal, the nucleic acid sequence being operably linked to a tetracycline-regulatable promoter, as required by claim 14 as amended. Specifically, the Cooke et al. reference does not teach successful tet-regulated expression of Nef in T cell or macrophage cell lines. In fact, the reference *teaches away from such tet-regulated expression*. For example, in column 1 of the abstract the reference states:

> "Tetracycline-regulated Nef expression was achieved in HeLa cells *but could not be established in human T cell lines*. Jurkat E6-1 T cell and RAW264.7 murine macrophage cell lines expressing a regulated nef gene were generated using a system in which Nef expression was controlled by a mutated version of the heavy metal-indicible human metallothionein IIA promoter." (Abstract, p. 381; emphasis added)

Also, on page 386, column 2, first full paragraph, the reference states:

> "An alternative gene expression system was sought *as a result of the inability of the tetracycline-responsive vectors to produce regulated Nef expression in T cells*." (emphasis added)

In view of the above, Applicants submit that the Cooke et al. reference does not teach an isolated leukocyte transformed with a nucleic acid sequence encoding a polypeptide which is immunogenic to a mammal, the nucleic acid sequence being operably linked to a tetracycline-

regulatable promoter, such that expression of the immunogenic polypeptide by the leukocyte is controlled by altering the concentration of tetracycline or an analog thereof to which the leukocyte is exposed after introduction to a mammal, as required by claim 14 as amended. Cooke et al. therefore does not anticipate claim 14 as amended, nor does it anticipate claim 16, which depends from claim 14. Further, the cancellation of claims 15 and 17 obviates this rejection of those claims.

Applicants respectfully request the withdrawal of the §102(a) rejection of amended claim 14 and claim 16 over Cooke et al.

Applicants further submit that the rejection over Cooke et al. applied to claims 14-17 does not apply to new claims 19 and 20. First, Cooke et al. does not teach introducing a cell to a mammal as required by new claim 19. Second, Cooke et al. does not teach introducing a cell to a mammal wherein prior to introduction of the cell into the mammal the expression of the polypeptide is inhibited in vitro, as also required by new claim 19. The reference also does not teach altering the concentration of tetracycline or an analog thereof to which the cell is exposed in the mammal, wherein expression of the polypeptide reaches a maximum level in the mammal after 2 days, as required by new claim 20. Because the Cooke et al. reference does not teach any of these elements required by new claims 19 and 20, the claims are not anticipated by the reference.

Rejections under 35 U.S.C. §103:

A. Cooke et al.

Claims 1-3 and 18 are rejected under 35 U.S.C. §103 as allegedly obvious over Cooke et al. The Office Action states that Cooke et al. teaches transfecting human T cell lines and mouse macrophages with a vector encoding *nef* operably linked to a CMV/tetracycline operator promoter and a vector encoding tTA. The Office Action states that Cooke et al. does not teach administering the cells to a mammal or altering the tet concentration, but that the reference "suggests administering the cells to a mammal and altering the tet concentration (page 390, col. 1, last paragraph; page 390, col. 2) which is all that is required of the claim." Applicants respectfully disagree.

First, as discussed above, Applicants submit that Cooke et al. does not teach the successful tet-regulated expression of Nef in T cells, macrophages, or any leukocyte. In fact, as

discussed above, Cooke et al. teaches away from the expression of Nef in leukocytes using a tet-regulated expression construct. The reference therefore does not teach or suggest introducing into a leukocyte a nucleic acid coding sequence operably linked to a tetracycline-operator sequence and a sequence encoding a tetracycline-sensitive DNA-binding expression-regulating polypeptide and altering the concentration of tetracycline or an analog thereof to which the leukocyte is exposed, so as to regulate expression of the coding sequence, as required by claim 18. As such, the Cooke et al. reference cannot render any of claims 2, 3 or 18 obvious, because each of these claims requires tet-regulatable expression in a leukocyte.

Second, Applicants submit that the passages of the Cooke et al. reference cited by the Office Action as allegedly providing a suggestion to administer the cells to an animal do not make such a suggestion. Page 390, column 1, last paragraph relates to in vivo studies with HIV and SIV, which showed reduced infectivity and cytopathicity of nef-deleted viruses. The reference states:

> "Nef may therefore possess an intrinsic cytopathogenicity that could be a contributory factor in the development of AIDS. This cytopathogenicity may be related to the ability of Nef to modulate cell proliferation and activation. The cell lines described in the current study will provide important reagents for further investigations of this aspect of Nef function."

Applicants submit that this passage may suggest the usefulness of the cell lines, but it does not in any way suggest introducing a cell to a mammal, as recited by claim 1.

The cited passage on page 390, column 2 also does not suggest administering cells or cell lines taught by the Cooke et al. reference to an animal. The cited passage relates to the instability of the expression from retrovirally-introduced nef constructs in cultured cells. The reference states:

> "Furthermore, the ability of these vectors to provide regulated expression allows detailed analysis of dose dependency and kinetics of Nef function(s). Consequently, these regulated vectors represent a significant advance from the use of constititive and retroviral vecotrs for generating cell lines expressing Nef. It is also anticipated that both the tetracycline- and heavy metal-inducible vector systems will provide useful vehicles for achieving regulated expression of other cytotoxic proteins."

Applicants submit that, again, this passage may suggest the usefulness of the cell lines, but it in no way suggests the administration of the cell lines to animals. The entire context of both cited passages is cells in culture. Applicants submit that neither of these passages, alone or together provides any suggestion to introduce cells to a mammal. As such, the Cooke et al. reference cannot render obvious any claim, e.g., claim 1 or its dependents, which requires such introduction.

Finally, Cooke et al. does not teach or suggest introducing into a mammal that has made an immune response to a polypeptide a cell comprising a vector comprising a nucleic acid encoding that polypeptide, operably linked to a tetracycline-regulatable promoter, as required by claim 1 as amended. Because the reference does not teach or suggest the introduction of any cell or construct encoding a polypeptide to which the mammal has made an immune response, Cooke et al. cannot render obvious any claim, e.g., claim 1, which has such a requirement.

In view of the above, Applicants respectfully request the withdrawal of the §103 rejection of claims 1-3 and 18 over Cooke et al.

Applicants further submit that new claims 19 and 20 are not obvious over Cooke et al. First, as discussed above, Cooke et al. does not teach or suggest introducing cells bearing a tet-regulated construct to a mammal.

Second, not only does the reference not teach or suggest such introduction, it does not teach or suggest introducing a cell to a mammal wherein prior to introduction of the cell into the mammal the expression of the polypeptide is inhibited in vitro, as required by new claim 19. The reference also does not teach or suggest altering the concentration of tetracycline or an analog thereof to which the cell is exposed in the mammal, wherein expression of the polypeptide reaches a maximum level in the mammal after 2 days, as required by new claim 20. Because the Cooke et al. reference does not teach any of these elements required by new claims 19 and 20, the claims are not rendered obvious by the reference.

B. Hoffmann et al.:

Claims 1-3, 13 and 18 are rejected as allegedly obvious over Hoffmann et al. The Office Action states that Hoffmann et al. "teaches transfecting lymphocytes with a retroviral vector encoding LacZ operatively linked tot he tet operator and also encoding the tetR-VP16," but that "Hoffmann does not expressly teach administering the cells to a mammal." The Office Action

concludes that it would have been obvious to one of skill in the art to administer the cells of Hoffmann to a mammal because Hoffmann suggests delivering the cells to a mammal. Applicants respectfully disagree.

First, as discussed above in relation to the §102 rejections over the Hoffmann et al. reference, Hoffmann et al. does not teach transfecting leukocytes, but rather, teaches transfecting muscle progenitor cells. Because the reference does not teach or suggest the transfection of leukocytes, it cannot render obvious claims requiring transfection of a leukocyte, e.g., claims 2, 3 and 18.

Second, with regard to claim 1, Applicants submit that Hoffmann et al. does not teach or suggest introducing, into a mammal that has made an immune response to a polypeptide, a cell comprising a vector comprising a nucleic acid encoding the polypeptide, operably linked to a tetracycline-regulatable promoter, and altering the concentration of tetracycline or an analog thereof to which the cell is exposed, as required by claim 1 as amended. Specifically, Hoffmann et al. does not teach or suggest introducing a tet-regulated vector construct into a mammal that has made an immune response to said immunogenic polypeptide encoded by the construct. Because the reference neither teaches nor suggests such a step, Hoffmann et al. cannot render obvious any claim requiring that step. Applicants therefore submit that claim 1 is not obvious over Hoffmann et al., and respectfully request that this §103 rejection be withdrawn.

Applicants further submit that the Hoffmann et al. reference does not render obvious new claims 19 and 20. As stated in the Office Action, Hoffmann et al. does not teach administering a cell to a mammal. Not only does the reference not teach such administration, it does not teach or suggest introducing a cell to a mammal wherein prior to introduction of the cell into the mammal the expression of the polypeptide is inhibited in vitro, as required by new claim 19. The reference also does not teach or suggest altering the concentration of tetracycline or an analog thereof to which the cell is exposed in the mammal, wherein expression of the polypeptide reaches a maximum level in the mammal after 2 days, as required by new claim 20. Because the reference does not teach either of these elements required by new claims 19 and 20, the claim are not rendered obvious by the Hoffmann et al. reference.

In view of the above amendments and remarks, Applicants submit that all issues relevant to patentability that were raised by the Office Action have been addressed. Reconsideration of the claims is respectfully requested.

Respectfully submitted,

Date 6/22/01

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Version marked to show changes:

Changes to the Abstract (page 1):

Abstract of the Disclosure

Disclosed is a method of regulating the expression in a human or animal subject of a nucleic acid sequence encoding a polypeptide which is immunogenic in the subject; the method [comprising] entailing introducing into a mammal a cell containing the nucleic acid sequence encoding the immunogenic polypeptide,[said] the sequence being operably linked to a drug-regulatable promoter; and altering the concentration of regulatory drug to which the cell is exposed.

Changes to the Specification:

The changes to the section "Brief Description of the Drawings" on page 7, lines 9-17 are as follows:

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A and 1B are schematic representations of nucleic acid constructs referred to in the example below[;].

[Figures 2A, 2B and 3 show representative FACS data;]

Figure 2 shows representative results confirming the regulationof the chTCR gene expression by tetracycline analogs. In Figure 2A, stable transfected uncloned JLAV12S (left hand side) and JN3S Jurkat (right hand side) cell populations were cultured for 48 hours in tetracycline-free medium (CM, upper row of panels) or in the presence of 1 μg/ml of Tet (broken line) or Dox (solid line) (lower row of panels) and the surface expression of chTCRs was examined after staining with FITC-conjugated goat antisera to mouseλ light chain. Figure 2B shows a timecourse of inactivation of chTCR gene expression in JLAV12S cells zero hours (top left), 8 hours (top right), 12 hours (bottom left) or 24 hours (bottom right) after addition of Dox at 1 μg/ml. In both Figures 2A and 2B negative controls (FITC-conjugated goat antisera to mouse IgG) are overlaid (filled curve). The fluorescence channel number is plotted along the x axis, and the y axis represents the relative cell number.

Figure 3 shows the results of dose response curves of gene repression determined using different concentrations of Tet or Dox. After 48 hours of treatment, the cells were harvested and the expression of the chTCR was studied by FACS analysis. Stably transfected uncloned (JLAV12S, left hand column) and cloned (1F5, middle column; and 2E11, right hand column)

Jurkat cell populations were cultured for 48 hours in the presence of different concentrations (0 ng/ml top row, 0.1 ng/ml second row, 1 ng/ml third row, and 10 ng/ml bottom row) of Tet (broken line) or Dox (solid line) and the surface expression of scFv-ξ molecules were examined. Negative controls (FITC-conjugated goat antisera to mouse IgG) are overlaid (filled curve). The fluorescence channel number is plotted along the x axis, and the y axis represents the relative cel number.

Figure 4 is a graph showing expression of a chimeric polypeptide (as a percentage of expression in control cells) against time[; and].

Figures 5A and 5B are bar charts showing the levels of IL-2 production (in picrograms/ml) by T lymphocytes exposed to various concentrations of tetracycline or the tetracycline analog, doxycycline.

Changes to the Claims

1. (Three times amended) A method of regulating the expression of a recombinant nucleic acid sequence encoding a polypeptide which is immunogenic in a mammal; the method comprising introducing, into a mammal that has made an immune response to said immunogenic polypeptide, a cell comprising a vector comprising a nucleic acid encoding said [a] immunogenic polypeptide, operably linked to a tetracycline-regulatable promoter; and altering the concentration of tetracycline or an analog thereof to which the cell is exposed so as to achieve in said mammal expression of said nucleic acid sequence as permitted in the presence or absence of tetracycline or an analog thereof.

5. (Twice amended) The method of claim 1 or claim 19, wherein prior to said introducing step, said mammal has circulating antibodies which react with said immunogenic polypeptide.

6. (Twice amended) The method of claim 1 or claim 19, wherein prior to said introducing step, said mammal has immunocompetent memory cells which are specific for said immunogenic polypeptide.

8. (Three times amended) The method of claim [7] 1 or claim 19, wherein expression of [the] said immunogenic polypeptide is inhibited in vitro by exposure of the cell to tetracycline or

an analog thereof, and wherein expression in the mammal is induced after 2 days following removal of exposure to tetracycline or an analog thereof.

9. (Three times amended) The method of claim [7] 1 or claim 19, wherein expression of [the] said immunogenic polypeptide is inhibited in vitro by the absence of tetracycline or an analog thereof and wherein expression in the mammal is induced to a maximum level after 2 days by administration of tetracycline or an analog thereof to the mammal.

13. (Twice amended) The method of claim 1 or claim 19, wherein said vector is a viral vector.

14. (Twice amended) An isolated [cell] leukocyte transformed with a nucleic acid sequence encoding a polypeptide which is immunogenic to a mammal, the nucleic acid sequence being operably linked to a tetracycline-regulatable promoter, such that expression of the immunogenic polypeptide by the [cell] leukocyte is controlled by altering the concentration of tetracycline or an analog thereof to which the [cell] leukocyte is exposed after introduction to a mammal.

16. A composition comprising a plurality of a [cell] leukocyte of claim 14 and a physiologically acceptable diluent.

18. A method of regulating the expression of a nucleic acid sequence encoding a heterologous polypeptide in a leukocyte, comprising introducing into the leukocyte the nucleic acid coding sequence operably-linked to a tetracycline-operator sequence, and a sequence encoding a tetracycline-sensitive DNA-binding expression-regulating polypeptide; and altering the concentration of tetracycline or an analog thereof to which the leukocyte is exposed, [so as to regulate] thereby regulating the expression of the coding sequence.